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Published in:
Obesity Reviews

DOI:
[10.1111/j.1467-789X.2009.00594.x](https://doi.org/10.1111/j.1467-789X.2009.00594.x)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Edens, M. A., Kuipers, F., & Stolk, R. P. (2009). Non-alcoholic fatty liver disease is associated with cardiovascular disease risk markers. *Obesity Reviews*, 10(4), 412-419. <https://doi.org/10.1111/j.1467-789X.2009.00594.x>

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Obesity Comorbidities

Non-alcoholic fatty liver disease is associated with cardiovascular disease risk markers

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Received 18 August 2008; revised 27 February 2009; accepted 2 March 2009

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Summary

Recognition of the link between non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) has boosted research in this area. The main objective of this paper is to review the literature on NAFLD in the context of CVD, focussing on underlying mechanisms and treatment. Besides excessive fatty acid influx, etiologic factors may include components of the metabolic syndrome, cytokines and mitochondrial dysfunction. NAFLD is associated with both hepatic and systemic insulin resistance. In the case of NAFLD, the liver overproduces several atherogenic factors, notably inflammatory cytokines, glucose, lipoproteins and coagulation factors, and factors increasing blood pressure. Intervention studies on diet and laparoscopic surgery revealed improvements of hepatic fat content and CVD risk profile. Pharmacological approaches with potential benefit have been developed as well, but effects are often confounded by weight change. NAFLD is associated with an increased CVD risk profile (and hepatic risk). In order to improve CVD risk profile, prevention and treatment of NAFLD seem advisable. However, well-designed intervention studies, randomized clinical trials and long-term follow-up studies are scarce.

Keywords: Cardiovascular disease, hepatic fat.

obesity reviews (2009) **10**, 412–419

Introduction

The global increase of overweight and obesity is alarming (1), as obesity is a risk factor for many diseases including cardiovascular disease (CVD) (2). Obesity has the highest CVD risk when fat is located in the abdominal region (3).

In the case of obesity accompanied by insulin resistance, triglycerides (TGs) are often excessively stored ectopically, i.e. in organs and muscles rather than in adipocytes. When TGs accumulate within hepatocytes (HCs; Fig. 1), a pathological condition usually referred to as fatty liver disease (FLD) will develop. FLD includes a wide spectrum, which can broadly be divided into steatosis and steatohepatitis (4). Non-alcoholic fatty liver disease (NAFLD) is used to describe FLD in a person who drinks no or little alcohol

prior to diagnosis. In the literature, the amount of ethanol allowed for the diagnosis of NAFLD varies greatly but is maximally 20 g d⁻¹ for women and 30 g d⁻¹ for men. The prevalence of NAFLD in the general adult western population is relatively high, i.e. 20% (5,6); whereas, the prevalence of total FLD, including both NAFLD and alcoholic FLD, is approximately 30% (5). In obese non-diabetic western adults, the prevalence of NAFLD ranges from 80.4% to 97.9% (7,8).

Although there is a hepatic risk for patients with NAFLD, notably cirrhosis (9) and hepatocarcinoma (10), the CVD risk for patients with NAFLD may be higher (10). Few studies have revealed evidence of an association between NAFLD and early CVD markers (11), CVD events (12,13) and CVD mortality (10). Follow-up of patients with

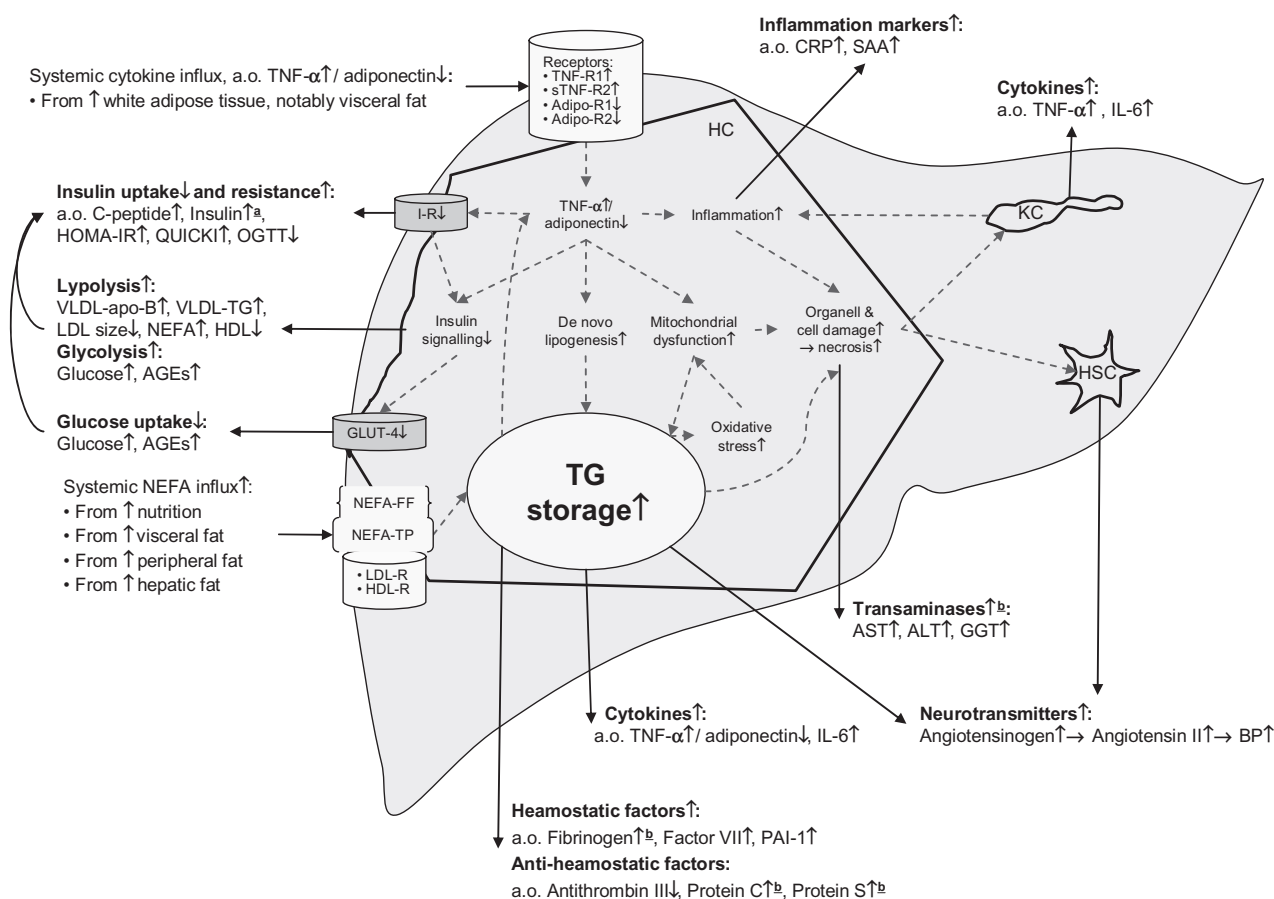


Figure 1 Model on the cardiovascular risk of non-alcoholic fatty liver disease. ↑, increase; ↓, decrease; ^a, dependent on pancreatic β -cell function; ^b, may decrease in advanced NAFLD; Adipo-R1, adiponectin receptor 1; Adipo-R2, adiponectin receptor 2; AGEs, advanced glycation endproducts; GLUT-4, glucose transporter 4; HDL-R, high-density lipoprotein receptor; I-R, insulin receptor; LDL-R, low-density lipoprotein receptor; NEFA-FF, non-esterified fatty acid flip-flopping; NEFA-TP, non-esterified fatty acid transport protein; SAA, serum amyloid A; sTNF-R2; soluble tumour necrosis factor- α receptor 2; TNF-R1; tumour necrosis factor- α receptor 1.

NAFLD showed a higher incidence of CVD compared with controls (10,13). A study by Hamaguchi *et al.* revealed that NAFLD was an independent predictor, even stronger than the metabolic syndrome, for first time CVD events (13).

The recognition of the CVD risk of NAFLD has boosted research in this area during the recent years. We have reviewed the literature on NAFLD in the context of CVD risk profile markers. In this paper, after a short description of the aetiology of NAFLD, we provide an overview of the cardiovascular risk of NAFLD. Finally, the potential need for prevention and treatment of NAFLD in order to improve CVD risk profile is addressed.

Aetiology

The aetiology of the hepatic lipid imbalance underlying the pathophysiology of NAFLD has been increasingly unravelled. Besides excessive non-esterified fatty acid influx, mediating factors may include: (i) components of

the metabolic syndrome (14,15); (ii) cytokines (16–24) and (iii) mitochondrial dysfunction (25) (Fig. 1). A study on the incidence of NAFLD suggested that the metabolic syndrome precedes NAFLD (14), with insulin resistance as a cornerstone (15). Animal models, reviewed by Diehl *et al.* (16), revealed involvement of the cytokine tumour necrosis factor- α (TNF- α) and its possible antagonist adiponectin (17) in the pathogenesis of NAFLD. This is reinforced by cross-sectional studies showing changes in mRNA of both TNF- α receptors (increase) and adiponectin receptors (decrease) in NAFLD (18–20). TNF- α , recently reviewed by Ryden and Arner, may have numerous mediating actions, among others increasing insulin resistance and inhibiting fatty acid oxidation (22). Several animal studies on adiponectin, recently reviewed by Lafontan and Viguerie, suggest opposite actions of adiponectin on energy metabolism, i.e. increasing insulin sensitivity and stimulation of fatty acid oxidation (23). Additionally, adiponectin might contribute to inhibition

of lipogenesis (24). Polymorphisms in the gene encoding adiponectin receptor 1 are associated with the presence of high hepatic fat content (and insulin resistance) (21). Mitochondrial dysfunction (in non-alcoholic steatohepatitis [NASH]), recently reviewed by Begriche *et al.*, can be caused by oxidative stress, and may result in TG accumulation and eventually cell death, i.e. necrosis (25).

Fatty liver-derived cardiovascular disease risk factors

The liver secretes numerous CVD risk factors, notably cytokines, glucose, lipoproteins, coagulation factors and factors increasing blood pressure. In the case of NAFLD, production of several of these risk factors is altered (Fig. 1).

Inflammation

In both patients with non-alcoholic steatosis (NAS) and NASH, analysis of liver biopsies revealed hepatic distribution (mRNA) of the inflammatory cytokine TNF- α with its receptors (18,20) and the anti-inflammatory adipocytokine adiponectin with its receptors (20). As suggested by animal models, the increased amount of fatty acids present in the case of NAFLD may mediate hepatic production of TNF- α , causing increased levels of systemic TNF- α (26). Upon HC damage activated liver-specific macrophages 'Kupffer Cells' will secrete more cytokines into the blood, among others TNF- α (18) and interleukin 6 (IL-6) (16,27,28). TNF- α and IL-6 are considered to induce hepatic production of the acute phase protein 'C-reactive protein' (CRP) (29).

Hepatic expression of TNF- α mRNA is significantly higher in patients with NASH compared with NAS (18,20), and hepatic expression of adiponectin mRNA is significantly lower in patients with NASH compared with NAS (20). Systemic TNF- α concentration is significantly elevated in both patients with NAS and NASH (19). Both hepatic IL-6 mRNA and systemic IL-6 concentration are elevated in patients with NAS and the highest in NASH (28). Elevated CRP is present in 25% of controls compared with 60% of NAFLD patients ($P = 0.003$) (30). Fasting adiponectin concentration predicts hepatic fat content (31,32) and is significantly lower in both patients with NASH and NAS compared with controls (19,32) for both men and women (19). Fasting adiponectin is inversely correlated with hepatic fat content in healthy non-diabetic subjects (31,33) but non-significantly in patients with type 2 diabetes mellitus ($n = 10$) (31). TNF- α , IL-6 and CRP may contribute to the inflammatory CVD milieu, predisposing to atherosclerosis and CVD (34–36). On the contrary, studies in both humans and animals (23,37) have revealed both anti-inflammatory (23) and antithrombotic (37) properties of adiponectin, enabling a protective association between adiponectin and CVD (38).

In addition to a direct predisposition to atherosclerosis, cytokines may have an indirect effect as well, as cytokines may mediate insulin resistance (Fig. 1). Animal models (22,39) and both *in vitro* (40) and *in vivo* (41) human studies provided evidence that not fat accumulation itself but fat-derived cytokines play a role in (obesity-related) insulin resistance. The pleiotropic cytokine TNF- α interferes with the hepatic insulin receptor and the intra-hepatocellular insulin signalling cascade (22,40), causing both hepatic and systemic insulin resistance (22,40). Additionally, administration of human recombinant TNF- α in human cancer patients resulted in increased levels of very low-density lipoprotein (VLDL) and TG and decreased levels of high-density lipoprotein (HDL) (41), which are features of diabetic dyslipidaemia (42).

Hyperglycaemia and diabetic dyslipidaemia

In the healthy situation, insulin stimulates hepatic and peripheral glucose uptake and suppresses hepatic glucose production. In patients with NAFLD, hepatic glucose uptake may be less effectively stimulated by insulin, contributing to elevated plasma glucose concentrations (40,43). Moreover, hepatic glucose production might be less effectively suppressed by insulin (40) but not in non-diabetic NAFLD patients (43,44).

Hepatic fat content correlates positively with fasting glucose (45), glucose levels after an oral glucose tolerance test (46), fasting C-peptide (45), fasting insulin (45) and insulin resistance by homeostatic model assessment (31). In the general population, the odds ratio of NAFLD compared with normal liver is 9.1 for hyperglycaemia (6). Additionally, the odds ratios of NAFLD, compared with normal liver, increase with increasing insulin quartile (4.2, 5.9 and 20.0 for the second, third and fourth quartile respectively) and insulin resistance by homeostatic model assessment quartile (2.3, 4.4 and 16.7 for the second, third and fourth quartile respectively) (6). Plasma glucose and its advanced glycation endproducts are considered atherogenic (47,48) and predispose to CVD (49).

In the healthy situation, insulin suppresses hepatic production and secretion of VLDL (50). In patients with NAFLD, VLDL secretion is less effectively suppressed by insulin, causing increased systemic VLDL-TG concentrations (31). Hepatic fat content correlates positively with VLDL₁-TG and VLDL₁-apolipoprotein-B secretion rates (31). Besides the presence of NAFLD, the altered hepatic lipid composition present in the case of NAFLD (51,52) might play a role in both altered VLDL secretion rates and altered lipoprotein composition. An *in vitro* study suggested that the presence of different types of fatty acids in the liver results in both different VLDL-apolipoprotein-B secretion rates and lipoprotein composition (53).

In patients with NAFLD, VLDL concentration is increased, VLDL particles are larger, small dense LDL particles predominate, whereas large HDL particle concentration is decreased (54). Hepatic fat content correlates inversely with fasting HDL cholesterol concentration (45). In the general population, the odds ratios of NAFLD compared with normal liver are 6.3 for low-HDL cholesterol concentration and 3.5 for hypertriglyceridaemia (6). Systemic lipids, i.e. TGs (48,55) and cholesterol (HDL excluded) (47,55), have been considered atherogenic and predispose to CVD (48,55).

Coagulation

Many coagulation factors are synthesized by HCs (56,57). The limited data on the association between obesity-independent markers of NAFLD, among others coagulation factors, has recently been reviewed by Kotronen and Yki-Jarvinen (45). In patients with NAFLD, the liver overproduces several factors (Fig. 1), of which plasminogen activator inhibitor-1 has direct atherogenic effects (47). However, many factors (fibrinogen, protein C and protein S) are increased in NAS but tend to be lower in NASH (58). This may suggest that initially increased factors will decrease while NAS progresses to advanced NASH. In advanced liver disease, bleeding problems are well known to occur (56,57).

Blood pressure

HCs produce angiotensinogen (59,60), a precursor of angiotensin II. Upon HC damage activated Hepatic Stellate Cells even synthesize and secrete mature angiotensin II (61). Angiotensin II is a major pro-atherogenic and vasoconstrictive peptide/neurotransmitter (47,61), considered to predispose to elevated blood pressure (59–61) and possibly CVD (62). Both systolic (only in females) and diastolic blood pressures correlate with hepatic fat content (45). In the general population, univariate odds ratios of NAFLD, compared with normal liver, are 2.0 for systolic hypertension and 1.7 for diastolic hypertension (6).

Prevention and treatment

As obesity (1), including childhood obesity (63), has been increasing in the general population, an earlier peak prevalence of NAFLD and an increased CVD risk profile may be expected in the future, delineating the need for prevention.

Once NAFLD is present, treatment seems advisable to improve CVD risk profile and hepatic risk. Prevention and treatment trials of NAFLD should focus on etiologic factors, notably systemic non-esterified fatty acid influx, components of the metabolic syndrome, cytokines and mitochondrial dysfunction (Fig. 1). Several studies have

investigated the effect of lowering hepatic fat content on improving CVD risk profile markers; however, no longitudinal studies on lowering CVD events have been performed yet.

For an overview on diagnosis modalities for NAFLD, the reader is referred to recent review papers (45,64). As serum levels of transferases are of limited use (45,64,65), the intervention studies included in Table 1 have been limited to studies with a diagnosis by histology or imaging.

Dietary (plus exercise) and laparoscopic surgery interventions (3–15 months) revealed that overall loss of adipose tissue, determined by a decreased body mass index (BMI) (2.6–18.2 BMI points), promotes loss of hepatic fat content and results in an improved CVD risk profile (66–70). This suggests that weight loss should be pursued in patients with NAFLD.

Patients unable to lose weight or non-overweight patients might benefit from drug treatment. However, no medication is currently licensed for NAFLD treatment even though some have shown potential benefit. Additionally, as weight loss is associated with an improvement of NAFLD (66–70), statistical adjustment for the amount of weight change as potential confounder (Table 1) should be considered when determining true effects of pharmacological interventions. For an overview on potential treatment modalities, the reader is referred to recent review papers (45,64).

Some of the most often prescribed medicine are based on improving CVD risk profile (components of the metabolic syndrome) by acting on hepatic lipid metabolism, e.g. statins and fibrates (71,72) and by acting on hepatic glucose metabolism, e.g. metformin (73). Although often prescribed, studies on statins (74), fibrates (75) and metformin (76,77) in the case of NAFLD are few, often accompanied by weight loss and inconclusive. Studies on the angiotensin II receptor antagonist losartan revealed beneficial histological results (74,78).

As the possible antagonists TNF- α and adiponectin (17) may be involved in both the aetiology of NAFLD (16–24) and the worsening of CVD risk profile (23,34–38), TNF- α -lowering in NAFLD might be beneficial to improve both NAFLD and CVD risk profile, as suggested by studies on pentoxifylline (74,79).

Conclusion

The NAFLD is associated with an increased CVD risk profile (and hepatic risk). In order to improve CVD risk profile, prevention and treatment of NAFLD seem advisable. However, well-designed intervention studies, randomized clinical trials and long-term follow-up studies are scarce.

Conflict of Interest Statement

No conflict of interest was declared.

Table 1 Selection of intervention studies on NAFLD and cardiovascular disease risk markers, limited to studies with a diagnosis by histology or imaging

Studied treatment modality	Patients	n	Length	HFC	Total NASH score/activity index	BMI	Reference
Before–after studies							
Moderate WL (2.6 BMI points) by diet	T2DM, obese*	8	1–3 months	↓	Not applicable	↓	(66)
Moderate WL (~2.7 BMI points) by diet	HFC > 5%, GDM	11	3–6 months	↓	Not applicable	↓	(67)
	HFC < 5%, GDM	12		↓	Not applicable	↓	
Severe WL (10.4 BMI points) by diet	Morbidly obese*	41	9 months	↓	–	–	(69)
Severe WL (17 BMI points) by laparoscopic surgery	NAFLD	70	15 months	↓	↓	↓	(70)
Atorvastatin (10 mg)	NASH, ↑lipids	10	9 months	↓	No change	No change	(74)
Metformin (maximum 2 g)	NASH, ↑ALT	17	12 months	↓	↓	↓	(76)
Losartan (50 mg)	NASH, hypertension	12	9 months	↓	↓	No change	(74)
Pentoxifylline (2 × 400 mg)	NASH, T2DM	13	9 months	↓	↓	No change	(74)
Pentoxifylline (3 × 400 mg)	NASH, ↑ALT	9	12 months	–	↓	No change#	(79)
Non-randomized controlled trials							
Moderate WL (3 BMI points) by diet/exercise vs. no diet/exercise	NAFLD, AFLD*	25	3 months	↓	–	↓	(68)
Ursodeoxycholic acid (13–15 mg kg ⁻¹) vs. clofibrate (2 × 1 g)	NASH, Cholelithiasis NASH, ↑TG	40	12 months	↓	–	No change	(75)
n-3 polyunsaturated fatty acids ethyl ester (1 g) vs. no n-3 polyunsaturated fatty acids ethyl ester	NAFLD*	56	6–12 months	↓	Not applicable	No change	(80)
Randomized clinical trials							
Rosiglitazone (2 × 4 mg) vs. metformin (2 × 1 g)	T2DM	20	4 months	↓	Not applicable	↑	(77)

*Ethanol intake unreported or higher than allowed for NAFLD.

#P ≤ 0.1.

↑, significant increase and/or significantly inferior; ↓, significant decrease and/or significantly superior; AFLD, alcoholic fatty liver disease; ALT, alanine aminotransferase; BMI, body mass index; GDM, gestational diabetes mellitus; HFC, hepatic fat content; n, amount of subjects who had both pre- and post-measurements; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; TGs, triglycerides; WL, weight loss.

Acknowledgement

F. K. is supported by EU Grant Hepadip (No. 018734).

References

- Friedman JM. Obesity in the new millennium. *Nature* 2000; **404**: 632–634.
- Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999; **341**: 1097–1105.
- Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P Jr., Razak F, Sharma AM, Anand SS. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet* 2005; **366**: 1640–1649.
- Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413–1419.
- Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, Schminke U, Kessler C, John U. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. *World J Gastroenterol* 2005; **11**: 1848–1853.
- Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44–52.
- Venturi C, Zoppini G, Zamboni C, Muggeo M. Insulin sensitivity and hepatic steatosis in obese subjects with normal glucose tolerance. *Nutr Metab Cardiovasc Dis* 2004; **14**: 200–204.
- Colicchio P, Tarantino G, del Genio F, Sorrentino P, Saldalamacchia G, Finelli C, Conca P, Contaldo F, Pasanisi F. Non-alcoholic fatty liver disease in young adult severely obese non-diabetic patients in South Italy. *Ann Nutr Metab* 2005; **49**: 289–295.
- Clark JM, Diehl AM. Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. *JAMA* 2003; **289**: 3000–3004.

10. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmquist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865–873.
11. Targher G. Non-alcoholic fatty liver disease, the metabolic syndrome and the risk of cardiovascular disease: the plot thickens. *Diabet Med* 2007; **24**: 1–6.
12. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, Zenari L, Falezza G. Nonalcoholic Fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes* 2005; **54**: 3541–3546.
13. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, Kawahito Y, Yoshida N, Suetsugu A, Kato T, Okuda J, Ida K, Yoshikawa T. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J Gastroenterol* 2007; **13**: 1579–1584.
14. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, Omatsu T, Nakajima T, Sarui H, Shimazaki M, Kato T, Okuda J, Ida K. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* 2005; **143**: 722–728.
15. Qureshi K, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 3540–3553.
16. Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005; **54**: 303–306.
17. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8**: 731–737.
18. Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34**: 1158–1163.
19. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46–54.
20. Kaser S, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 2005; **54**: 117–121.
21. Stefan N, Machicao F, Staiger H, Machann J, Schick F, Tschrirter O, Spieth C, Weigert C, Fritsche A, Stumvoll M, Haring HU. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 2005; **48**: 2282–2291.
22. Ryden M, Arner P. Tumour necrosis factor-alpha in human adipose tissue – from signalling mechanisms to clinical implications. *J Intern Med* 2007; **262**: 431–438.
23. Lafontan M, Viguerie N. Role of adipokines in the control of energy metabolism: focus on adiponectin. *Curr Opin Pharmacol* 2006; **6**: 580–585.
24. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; **112**: 91–100.
25. Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion* 2006; **6**: 1–28.
26. Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology* 2004; **40**: 185–194.
27. Choi S, Diehl AM. Role of inflammation in nonalcoholic steatohepatitis. *Curr Opin Gastroenterol* 2005; **21**: 702–707.
28. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372–1379.
29. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med* 2002; **252**: 283–294.
30. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1045–1050.
31. Adiels M, Taskinen MR, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, Vehkavaara S, Hakkinen A, Olofsson SO, Yki-Jarvinen H, Boren J. Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* 2006; **49**: 755–765.
32. Targher G, Bertolini L, Rodella S, Zoppini G, Scala L, Zenari L, Falezza G. Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2006; **64**: 679–683.
33. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, Fredriksson J, Yki-Jarvinen H. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* 2004; **47**: 1360–1369.
34. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107**: 499–511.
35. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; **351**: 2599–2610.
36. Lizardi-Cervera J, Chavez-Tapia NC, Perez-Bautista O, Ramos MH, Uribe M. Association among C-reactive protein, Fatty liver disease, and cardiovascular risk. *Dig Dis Sci* 2007; **52**: 2375–2379.
37. Kato H, Kashiwagi H, Shiraga M, Tadokoro S, Kamae T, Ujii H, Honda S, Miyata S, Ijiri Y, Yamamoto J, Maeda N, Funahashi T, Kurata Y, Shimomura I, Tomiyama Y, Kanakura Y. Adiponectin acts as an endogenous antithrombotic factor. *Arterioscler Thromb Vasc Biol* 2006; **26**: 224–230.
38. Dekker JM, Funahashi T, Nijpels G, Pilz S, Stehouwer CD, Snijder MB, Bouter LM, Matsuzawa Y, Shimomura I, Heine RJ. Prognostic value of adiponectin for cardiovascular disease and mortality. *J Clin Endocrinol Metab* 2008; **93**: 1489–1496.
39. Grefhorst A, Hoekstra J, Derks TG, Ouwens DM, Baller JF, Havinga R, Havekes LM, Romijn JA, Kuipers F. Acute hepatic steatosis in mice by blocking beta-oxidation does not reduce insulin sensitivity of very-low-density lipoprotein production. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G592–G598.
40. Gupta D, Varma S, Khandelwal RL. Long-term effects of tumor necrosis factor-alpha treatment on insulin signaling pathway in HepG2 cells and HepG2 cells overexpressing constitutively active Akt/PKB. *J Cell Biochem* 2007; **100**: 593–607.
41. Sherman ML, Spriggs DR, Arthur KA, Imamura K, Frei E III, Kufe DW. Recombinant human tumor necrosis factor administered as a 5-day continuous infusion in cancer patients: phase I

- toxicity and effects on lipid metabolism. *J Clin Oncol* 1988; **6**: 344–350.
42. Adiels M, Boren J, Caslake MJ, Stewart P, Soro A, Westerbacka J, Wennberg B, Olofsson SO, Packard C, Taskinen MR. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1697–1703.
43. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002; **87**: 3023–3028.
44. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; **48**: 634–642.
45. Kotronen A, Yki-Jarvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2008; **28**: 27–38.
46. Thamer C, Machann J, Haap M, Stefan N, Heller E, Schnodt B, Stumvoll M, Claussen C, Fritsche A, Schick F, Haring H. Intrahepatic lipids are predicted by visceral adipose tissue mass in healthy subjects. *Diabetes Care* 2004; **27**: 2726–2729.
47. Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of atherosclerosis. *Am J Cardiol* 2007; **99**: 6B–14B.
48. Heine RJ, Dekker JM. Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. *Diabetologia* 2002; **45**: 461–475.
49. Goff DC Jr, Gerstein HC, Ginsberg HN, Cushman WC, Margolis KL, Byington RP, Buse JB, Genuth S, Probstfield JL, Simons-Morton DG. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Am J Cardiol* 2007; **99**: 4i–20i.
50. Sparks JD, Sparks CE. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *J Biol Chem* 1990; **265**: 8854–8862.
51. Araya J, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, Poniachik J. Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)* 2004; **106**: 635–643.
52. Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081–1090.
53. Mitmesser SH, Carr TP. Trans fatty acids alter the lipid composition and size of apoB-100-containing lipoproteins secreted by HepG2 cells. *J Nutr Biochem* 2005; **16**: 178–183.
54. Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Orvos JD, Caprio S. Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. *Diabetes Care* 2007; **30**: 3093–3098.
55. Gotto AM Jr. Triglyceride: the forgotten risk factor. *Circulation* 1998; **97**: 1027–1028.
56. Peck-Radosavljevic M. Review article: coagulation disorders in chronic liver disease. *Aliment Pharmacol Ther* 2007; **26**(Suppl. 1): 21–28.
57. Thachil J. Relevance of clotting tests in liver disease. *Postgrad Med J* 2008; **84**: 177–181.
58. Assy N, Bekirov I, Mejritsky Y, Solomon L, Szvalb S, Hussein O. Association between thrombotic risk factors and extent of fibrosis in patients with non-alcoholic fatty liver diseases. *World J Gastroenterol* 2005; **11**: 5834–5839.
59. Ron D, Brasier AR, Habener JF. Transcriptional regulation of hepatic angiotensinogen gene expression by the acute-phase response. *Mol Cell Endocrinol* 1990; **74**: C97–C104.
60. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 2001; **15**: 2727–2729.
61. Bataller R, Sancho-Bru P, Gines P, Lora JM, Al-Garawi A, Sole M, Colmenero J, Nicolas JM, Jimenez W, Weich N, Gutierrez-Ramos JC, Arroyo V, Rodes J. Activated human Hepatic Stellate Cells express the renin-angiotensin system and synthesize angiotensin II. *Gastroenterology* 2003; **125**: 117–125.
62. Silventoinen K, Magnusson PK, Neovius M, Sundstrom J, Batty GD, Tynelius P, Rasmussen F. Does obesity modify the effect of blood pressure on the risk of cardiovascular disease? A population-based cohort study of more than one million Swedish men. *Circulation* 2008; **118**: 1637–1642.
63. Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J Pediatr Gastroenterol Nutr* 2006; **43**: 413–427.
64. Preiss D, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. *Clin Sci (Lond)* 2008; **115**: 141–150.
65. Kunde SS, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. *Hepatology* 2005; **42**: 650–656.
66. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005; **54**: 603–608.
67. Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Hakkinen AM, Tamminen M, Teramo K, Yki-Jarvinen H. Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes* 2003; **52**: 701–707.
68. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997; **27**: 103–107.
69. Andersen T, Gluud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol* 1991; **12**: 224–229.
70. Mattar SG, Velcu LM, Rabinovitz M, Demetris AJ, Krasinskas AM, Barinas-Mitchell E, Eid GM, Ramanathan R, Taylor DS, Schauer PR. Surgically-induced weight loss significantly improves nonalcoholic fatty liver disease and the metabolic syndrome. *Ann Surg* 2005; **242**: 610–617.
71. Chalasani N. Statins and hepatotoxicity: focus on patients with fatty liver. *Hepatology* 2005; **41**: 690–695.
72. Paumelle R, Staels B. Cross-talk between statins and PPAR alpha in cardiovascular diseases: clinical evidence and basic mechanisms. *Trends Cardiovasc Med* 2008; **18**: 73–78.
73. Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: scientific review. *JAMA* 2002; **287**: 360–372.
74. Georgescu EF, Georgescu M. Therapeutic options in non-alcoholic steatohepatitis (NASH). Are all agents alike? Results of a preliminary study. *J Gastrointest Liver Dis* 2007; **16**: 39–46.
75. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate

in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology* 1996; **23**: 1464–1467.

76. Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; **100**: 1082–1090.

77. Tiikkainen M, Hakkinen AM, Korshennikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004; **53**: 2169–2176.

78. Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, Hasegawa T, Tokusashi Y, Miyokawa N, Nakamura K.

Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology* 2004; **40**: 1222–1225.

79. Satapathy SK, Sakhuja P, Malhotra V, Sharma BC, Sarin SK. Beneficial effects of pentoxifylline on hepatic steatosis, fibrosis and necroinflammation in patients with non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2007; **22**: 634–638.

80. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, Svegliati-Baroni G, Sofi F, Milani S, Abbate R, Surrenti C, Casini A. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. *Aliment Pharmacol Ther* 2006; **23**: 1143–1151.